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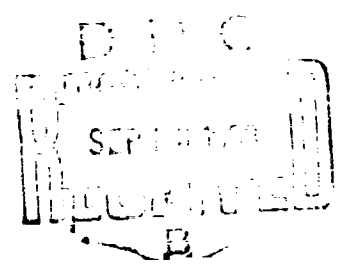

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Frederick, Maryland

IMMUNITY OF WHITE MICE TO EEE VIRUS

[Following is a translation of an article by Erich Traub of the West German Research Institute for Virus Diseases of Animals in Tübingen, in the German-language periodical, Zeitschrift für Immunitätsforschung (Journal of Immunity Research), Vol. 122, 1961, pages 229-238.]

[This is the seventh report of a series on the above subject. Its sub-title is, "Further Investigations of the Role of Interference in Cerebral Immunity."]

In the fourth and fifth papers of this series (9, 10) [numerals in parentheses refer to similarly numbered items in Bibliography at end] some clues were obtained indicating that the cerebral immunity of mice to EEE (eastern equine encephalomyelitis) virus did not depend solely on the local concentration of antibodies, but that interference played a certain part, at least during the first stage. The presence of an interferon in the brains of EEE-immune mice has nevertheless still remained to be proven indisputably in tissue culture tests. Attempts are therefore being made to demonstrate the presence of an interference phenomenon with the aid of a different virus.

Back in 1944 Schlesinger, Olitsky and Morgan (5) found in tests carried out with WEE (western equine encephalomyelitis) virus that experimental infection with about 1000LD_{50} led to a cerebral infection which followed an abortive course in vaccinated guinea pigs and mice. Subsequently the animals remained resistant to intracranial reinfection with a heterologous virus, for example, the agents causing EEE or herpetic stomatitis. In the opinion of this

researcher here was a case of "induced non-specific cellular resistance," evoked by the interference phenomenon. Schlesigner (4) in a later work no longer pays any attention to this observation. He rather looks upon the local concentration or formation of specific antibodies as the sole cause of the cerebral immunity of mice to the WEE-virus.

We attempted in the experiments described below to determine the existence of any condition of interference to VEE (Venezuelan equine encephalomyelitis) virus (1) which might be found in the brains of mice which had either been actively immunized with infectious EEE-virus (9) or which had been given a large dose of EEE-virus intracranially after passive immunization with specific highly immune serum (10). In the latter case the animals mostly became ill with encephalitis (7, 10) only after passage of a preliminary stage of latency lasting several weeks. The processes taking place within this latent period particularly aroused our interest.

Materials and Methods

Virus strains: Strain S 18888 (6) was again used as the EEE-virus. We also wish to thank the former Bureau of Animal Industry of the U.S. Department of Agriculture in Washington, D.C. for the VEE-virus. We do not know exactly through how many animals this strain has passed. The materials used for infection in both cases were 20 per cent brain suspensions both carried out in our institute (German Research Institute for Virus Diseases of Animals in Tübingen) by intracranial inoculations from one mouse to another.

Mice: In respect to the origin of the experimental mice used for our tests, reference is made to an earlier paper (6) on this subject. We used female mice about four months old for the active immunization with EEE-virus and we used females about seven weeks old for the passive immunization.

Active immunization with EEE-virus: The animals were given subcutaneously 0.2 ml. of a 10^{-1} or a 10^{-2} dilution of an infectious 20% mouse brain suspension. Subsequently 85% of the infected mice did not become ill while the remainder of the animals became infected with deadly encephalitis. In order to create the hypothetical condition of interference in the brain we injected a 10^{-1} or 10^{-3} virus dilution eleven days later intracranially into those animals which had not become ill. (See Table 1). After

injection of the 10^{-1} dilution 20-25 per cent of the mice became infected with deadly encephalitis. Other animals showed temporary encephalitic phenomena often after a short period of incubation. Central nervous system disturbances, for the most part more or less severe partial paralysis of the forefeet, persisted in a small number of the ill mice. After intracranial injection of the 10^{-3} virus dilution only very few animals became ill and none of them died.

Passive immunization with hyperimmune serum and intracranial experimental infection with EEE-virus: To this end we used hyperimmune serum from mice which had not fallen ill after subcutaneous injection and which had been hyperimmunized by nine subsequent virus injections (7). Each of the animals was given 0.2 ml. of immunization serum intravenously and three hours later they were given EEE-virus intracranially (10^{-1} dilution of an infectious 20 per cent mouse brain suspension). Afterwards about 10 per cent of the mice became ill with deadly encephalitis after a two to five day incubation period. Certain animals showed temporary encephalitis symptoms and lasting damage to the central nervous system remained in a few cases, while the great majority of the mice at first remained healthy. Many animals though became ill with encephalitis at a later time (See tables 2 and 3). The incubation period in these cases varied from 17 to 53 days.

Intracranial titration of VEE-virus: This took place at the times shown in the table useing 8 to 14 animals for each decimal solution. The type and number of checks carried out are also shown in the tables. The Reed and Muench (3) method was used to perform the assay (LD_{50}).

Tests and Results

Behavior of mice with active EEE-immunity to experimental intracranial infection with VEE-virus:

Table 1 shows the results of tests on mice which were actively immunized with EEE-virus in different ways. This table gives the VEE-virus intracranial titrations for the times shown. The dilutions shown refer to an infectious 20 per cent mouse brain suspension.

TABLE 1

Intracranial Titration of VEE-virus in Mice with Active Immunity to EEE

Experiment No.	Mouse group No.	Previous treatment with EEE-virus	Intracranial titration of VEE-virus: Days after first infection	Virus dilution	Mortality	Average degree of illness	LD_{50}
1	I	10^{-2} subcutaneously	17	10^{-5}	1/9	(0-5-10^{-7})	4.50
		10^{-1} intracranially		10^{-6}	0/8	0.7+	
		11 days later		10^{-7}	0/8		
	II	10^{-2} subcutaneously	17	10^{-5}	1/9	(10^{-5}-10^{-7})	5.00
		10^{-3} intracranially		10^{-6}	5/8	2.2+	
		11 days later		10^{-7}	2/8		
	III	- (Control Animals)	-	10^{-5}	9/9		6.79
				10^{-6}	8/8	(10^{-5}-10^{-7})	
				10^{-7}	3/8	3.2+	
				10^{-8}	0/8		
	IV	10^{-1} subcutaneously	31	10^{-1}	0/5	(10^{-1}-10^{-4})	1.00
		10^{-1} intracranially		10^{-2}	3/8		
		11 days later		10^{-3}	0/8	2.3+	
				10^{-4}	1/8		
2	V	10^{-1} subcutaneously	31	10^{-4}	10/10	(10^{-4}-10^{-7})	5.75
				10^{-5}	7/8		
				10^{-6}	2/8	3.1+	
				10^{-7}	2/8		

Table 1 Continued

Experiment No.	Mouse group No.	Previous treatment with EEE-virus	Days after first infection	Virus dilution	Mortality	Average degree of illness	LD_{50}
	VI	(Control Animals)		10^{-4}	10/10	(10^{-4}-10^{-7})	
				10^{-5}	8/8		
				10^{-6}	8/8	3.5+	7.00
				10^{-7}	4/8		
				10^{-8}	0/8		

*4+ = illness ending in death; 3+ = seriously ill but not ending in death; 2+ = moderately serious; 1+ = light illness; 0+ = no illness. The mice which did not become ill within the decimal dilutions represented between parentheses were also calculated in determining the average value in column headed "Average degree of illness".

As shown in Table 1, the mice in groups I and IV show the greatest resistance to VEE-virus after a first infection with a 10^{-1} dilution of EEE-virus, followed by the animals in Group II given 10^{-3} dilutions subcutaneously. The VEE-virus is so different from EEE-virus serologically that specific antibodies do not prevent a proper interpretation of the results from interference tests from being made (See also Table 3). Even if a remote serological relationship between the two viruses were to be found, it still could not explain such a high-level crosswise resistance as in mouse groups I and IV on the basis of effects produced by anitbodies. It is very probably then a case of an interference phenomenon. It is known of interference that it can be effective even against very large dosages of a heterologous virus (12).

Mutual Interference between EEE and VEE-viruses in Passively Immune Mice:

It was shown in an earlier work (10) that in passively immune mice there is generally an absence of any local appearance of antibodies remaining in the tissues if the animals are given a very large dosage of EEE-virus intracranially. The injected antiserum prevents formation of antibodies. The long delay in appearance of the illness undoubtedly does not solely depend on the effect of the artificially introduced antibodies (10). It can only be ex-

plained meaningfully by assuming the simultaneous appearance of a condition of interference. This hypothesis is tested for its validity in the following experiments by intracranialtitration of VEE-virus during the period of latency.

TABLE 2

Mutual Interference between EEE and VEE-viruses in the Brains of Passively Immunized Mice (Experiment No. 1)

Mouse group	Preliminary treatment	Intracranial titration of VEE-virus on the eleventh day after preliminary treatment with EEE-virus				Late or delayed-action illness after EEE in surviving mice	
		Virus dilution	Mortality	LD_{50}	Average time survived before death (days)	Quantity	Average incubation time (days)
A	EEE-hyperimmune serum intravenously; no EEE-virus (passively immunized control animals)	10^{-5}	8/8	7.23	8.3	-	-
		10^{-6}	8/8				
		10^{-7}	4/8				
		10^{-8}	2/8				
		10^{-9}	0/8				
B	EEE-hyperimmune serum intravenously; 10^{-1} EEE-virus dilution given intracranially three hours later	10^{-5}	5/10	5.66	11.3	0/5	-
		10^{-6}	4/8			0/4	-
		10^{-7}	2/8			3/6	34
		10^{-8}	0/8			7/8	30
		10^{-9}	0/8			6/8	29

Table 2 Continued

C	Untreated Control Animals	10^{-5}	8/8	7.71	7.9	-	-
		10^{-6}	8/8				
		10^{-7}	7/8				
		10^{-8}	3/8				
		10^{-9}	0/8				

The first experiments shown in Table 2 involved an interval of 11 days between the time of treatment with EEE-virus and titration of the VEE-virus. The arrangement of the experiments and their results can be seen from the table.

Significantly lower LD_{50} figures were obtained for the VEE-virus titrations in the mice in Group B which were given the 10^{-1} EEE-virus dilutions intracranially after previous treatment with hyperimmune serum than in those which had only been treated with immune serum and the control animals. In addition the time of survival before death set in averaged three days longer. But in the reverse direction, the interfering effect is unmistakable, because the animals in Group B which had been given the 10^{-5} and the 10^{-6} VEE-virus dilutions intracranially did not become ill after receiving EEE, whereas the great majority of those mice which had been injected with weaker Vee-virus dilutions fell ill at the later date. As in the previous experiments the illness always resulted in the deaths of the animals.

The difference between the lethal dosage titrations of the VEE-virus in the untreated animals and in the control animals treated only with immune serum (approx. 0.5 log) is to all appearances insignificant because in a repeat experiment (Table 3) in which the same hyperimmune serum was used it failed to make its appearance.

The experiment carried out in Table 3 involved a shortening of the period of time between treatment with EEE virus and the titration of EEE-virus to eight days. In addition a group of 10 mice given immune serum intravenously and EEE-virus intracranially but without receiving any VEE-virus was slaughtered.

The result of this second experiment was practically identical with that of the first experiment on Table 2. Once again there was no late appearance of illness subsequent to EEE when 10^{-5} and 10^{-6} VEE-virus dilutions had been given intracranially (Group E), while 87% of the remaining mice in this group came down with the illness at a

later date. This time, too, the disease ended in death in all cases.

TABLE 3

Mutual Interference between EEE and VEE-viruses in the Brains of Passively Immunized Mice (Experiment No. 2)

Mouse group	Preliminary treatment	Intracranial titration of VEE-virus on the eighth day after preliminary treatment with EEE-virus			Late or delayed-action illness after EEE in surviving mice		
		Virus dilution	Mortality	LD_{50}	Average time survived before death (days)	Quantity	Average incubation time (days)
D	EEE-hyperimmune serum intravenously; no EEE-virus (passively immunized control animals)	10^{-5}	8/8	7.79	6,5	-	-
		10^{-6}	8/8				
		10^{-7}	8/8				
		10^{-8}	3/8				
		10^{-9}	0/8				
E	EEE-hyperimmune serum intravenously; 10^{-5} EEE-virus dilution given i.c. three hours later	10^{-5}	5/15	6.00	9.3	0/9	-
		10^{-6}	5/8			0/3	-
		10^{-7}	3/8			4/5	33
		10^{-8}	0/8			8/8	33
		10^{-9}	0/8			7/8	30
		-	-	-	-	8/10	30

Table 3 Continued

F	Untreated control animals	10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9}	8/8 8/8 6/8 4/8 0/8	7.76	6,0	-	-

Discussion

In particular the results of the last described experiment unequivocally support the fact that the phenomenon being dealt with in this discussion is really one of interference. The performance of suitable checks excluded the presence of any antibody effect. It is, therefore, suggested that the results obtained in actively immune mice, too, (Table 1) be attributed to the interference phenomenon. These animals showed a surprisingly high degree of resistance to the heterologous VEE-virus. It should be assumed that they are protected even more against the homologous virus (11). The duration of the effect is unknown. It apparently lasts considerably longer than three weeks (See second experiment, Table 1). Nothing yet is known in detail about the mechanism of its action.

The cerebral interference effect appears to be particularly strong in those mice in which the EEE-virus has multiplied in the brain after intracranial injection of a large dosage (9) as for example in the cases of mouse groups I and IV in Table 1. There is a clear difference between these animals and those mice injected with 10^{-3} EEE-virus dilutions. In accordance with earlier findings (9) a multiplication of the virus in the brain would not be held to have occurred in the cases of all the animals. It is very improbable that such could have happened to the mice in group V (6) which received only a subcutaneous injection of EEE-virus. Nevertheless a slight degree of resistance was also to be noted in these animals, too, and its cause remains uncertain. One could imagine the existence of a general interferon effect. We hold that an antibody effect is not very probable because it is completely absent in the passively immune control animals in Table 3.

The fact that the later appearing illness can be prevented in the cases of the passively immunized mice after intracranial infection with a large dose of EEE-virus by means of a superinfection with a suitable dosage of

VEE-virus during the latent stage (see Group B in Table 2 and Group E in Table 3) is of particular interest in respect to the mechanism of inactivation of the virus in the brain. One can therefore achieve a life-saving therapeutic effect by utilizing the interference phenomenon, putting to use an otherwise dangerous virus. Perhaps, though, there may be other less dangerous viruses which fulfill the same purpose. It is improbable that this antagonistic effect depends upon the effect of antibodies. Earlier experiments have shown that passively immune mice generally do not form any antibodies of their own in the brain after intracranial experimental infection with EEE-virus. Neither should it be assumed then that the VEE-infection induces a local formation of EEE-antibodies. Schlesinger (4) has in fact determined that WEE-antibodies are formed in the brains of mice with partial immunity to the WEE-virus after intracranial infection with homologous virus, but not after an intracranial injection of EEE-virus. To all appearances then it is possible that small quantities of intracellularly located EEE-virus could be inactived during the course of the interference process even if the local formation of specific antibodies fails to occur.

It can be taken as certain that the interference phenomenon also plays a part in immunity to other animal viruses, for example like lymphocytic choriomeningitis in mice. It appears here to be the only immunity factor present in those immunologically tolerant animals which do not form specific antibodies (8). But even in non-tolerant immune mice it has a certain amount of importance (11). One should also mention foot-and-mouth disease wherein it appears that it is chiefly interference which is responsible for the fact that after a first infection with a serological virus type and reinfection with a second type only 58-80% of the animals come down with the disease again, while after reinfection with a third type only about 40-70% of the animals become sick again; there being no difference in which order the different types are given (13). These findings cannot be attributed to any serological relationship among the different virus types.

Summary

An attempt is made to explain the resistance of EEE-immune mice to intracerebral challenge with VEE-virus serologically different from the EEE-virus on the basis of an interference phenomenon.

When mice which had been passively immunized with homologous EEE-hyperimmune serum were shortly afterwards given intracranial experimental infection with a large dosage (about 10^{-6} LD) of EEE-virus and eight or ten days later were given intracranial decimal solutions of VEE virus, a mutual interference was found between the two serologically different virus strains. While 80% of control animals not infected with VEE virus became ill at a later time from EEE, those which were given superinfections intracranially with 10^{-2} to 10^{-3} LD_{50} of VEE virus did not show any EEE illness at a later date. An autosterilization of the brain evidently took place in the absence of any specific antibodies.

The significance of interference phenomena as a factor in immunity is discussed in connection with EEE and other animal virus diseases.

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